**pitkin**

Differential gene expression during development comprises establishment and maintenance of defined expression patterns. These epigenetic patterns of gene expression depend on alternative self-perpetuating higher-order chromatin states, which are achieved through balanced activities of repressing and actuating chromatin functions.

Genes encoding functions controlling higher-order chromatin structure in Drosophila have been identified in screens for dominant suppressor and enhancer mutations of position-effect variegation. In position-effect variegation (PEV), euchromatic regions become transcriptionally silenced after their relocation into a new intimate vicinity of pericentric heterochromatin. The >400 dominant PEV modifier mutations isolated (Reuter and Wolfe 1981; Sinclair et al. 1983; Wustmann et al. 1989; Dorn et al. 1993) have provided an enriched source for the identification of new genes affecting gene transcription and chromatin regulation (Grigliatti 1991; Reuter and Spierer 1992; Weiler and Wako-moto 1995). Molecular analysis of several of these PEV modifier genes in several cases proved involvement in various aspects of chromatin regulation. These loci encode heterochromatin-associated proteins like HP1 (James and Elgin 1986; Eisenberg et al. 1990, 1992), SU(VAR)3-7 (Reuter et al. 1990; Cleard et al. 1997), and SU(VAR)3-9 (Tschiertsch et al. 1994; Schotta and Reuter 2000), or chromatin proteins, which are also involved in transcriptional control of euchromatic loci like MODULO (Krejci et al. 1989; Perrin et al. 1998), MOD(MDG4) (Dorn et al. 1993; Gerasimova et al. 1995; Büchner et al. 2000), E2F (Seum et al. 1996), and the E(var) gene hel, which encodes a DEAD-box helicase (Eberl et al. 1997). The E(Pc) gene suppresses PEV and controls expression of homeotic and other genes (Sinclair et al. 1998). Other modifier loci encode enzymatic functions that affect modification of chromosomal proteins, e.g., Su(var)3-6, which encodes protein phosphatase PP1 (Baks et al. 1993), or Su(var)2-1, which affects histone H4 deacetylation (Dorn et al. 1986) and histone deacetylase RPD3 mutations (DeRubertis et al. 1996; Mottus et al. 2000).

Almost all of the dominant modifiers of PEV mutations were isolated on the basis of their suppressor or enhancer effect on white gene variegation associated with the In(1)wm1 rearrangement. The wm1 phenotype is very sensitive for the detection and genetic analysis of dominant PEV modifier mutations (Reuter et al. 1985).
In a certain number of eye primordial cells, heterochromatin spreads into neighboring euchromatin and the white locus in w^st becomes silenced by acquiring a more condensed and less accessible chromatin structure (Reuter and Szidonya 1983; Bovin and Dura 1998). It is likely that modification of chromatin structure in the euchromatic white gene region and/or flanking heterochromatin leads to suppression or enhancement of white variegation in w^st. Enhancer mutations result in excess heterochromatization, preventing expression of the white gene in almost all of the eye primordial cells whereas suppressor mutations allow white\textsuperscript{+} gene expression in almost all eye cells.

Most E(var) and Su(var) mutations are loss-of-function type mutations and the corresponding loci represent haplo-insufficient genes; a deletion for such a locus displays a dominant modifier effect on PEV indicating that two wild-type gene copies are required for normal function (Reuter and Spierer 1992). The haplo-dependent effect of PEV modifier genes implies that E(var) products are required for open chromatin conformation, whereas the products of Su(var) genes appear to be involved in chromatin condensation. However, not only juxtaposition of euchromatic and heterochromatic regions but also abnormal nuclear position of a gene or its inclusion into repeated arrays may cause gene silencing (cf. Henikoff 1997; Weiler and Wakimoto 1998).

To understand whether spreading of chromatin status, nuclear compartmentalization, or chromosome pairing represent nonmutually exclusive mechanisms for gene silencing in PEV, further understanding of the biochemical structure and molecular regulation of heterochromatic silencing complexes is required. In these studies gain-of-function type PEV modifier mutations like ptk\textsuperscript{disco} (ptn\textsuperscript{b}) might represent useful tools for genetic dissection of the unknown regulatory components of heterochromatic silencing complexes.

This article provides a combined genetic and developmental analysis of ptn\textsuperscript{b}, a gain-of-function type PEV enhancer mutation of Drosophila. Strong enhancement of PEV by ptn\textsuperscript{b} is accompanied by elevated spreading of heterochromatin-induced gene inactivation into euchromatic regions in all tested PEV rearrangements. In addition to its strong PEV enhancer effect, ptn\textsuperscript{b} induces repression of several mini-white transgenes located within euchromatin, which appears to be correlated with ectopic chromosomal distribution of heterochromatin protein SU(VAR)3-9.

ptn\textsuperscript{b} also induces dominant female sterility through a combination of maternal effect lethality and a zygotic lethal effect as early as cleavage cycle 1–3, which is accompanied by excess condensation of cleavage nuclei. Complete degeneration of egg primordia associated with condensation of nurse cell nuclei is found in ptn\textsuperscript{b}/Df ptn\textsuperscript{w}, ptn\textsuperscript{w}/ptn, ptn\textsuperscript{b}/ptn\textsuperscript{b}, and ptn/ptn females, indicating a requirement of the ptn gene during differentiation of female germ-line cells.

Our results imply a dominant negative nature of the ptn\textsuperscript{b} mutation; i.e., the ptn\textsuperscript{b}-encoded mutant gene product impedes the activities of the maternally provided wild-type counterpart at early embryogenesis. These data underline the importance of gain-of-function mutations for the study of chromatin regulation during germ-line development and early embryogenesis.

**MATERIALS AND METHODS**

**Origin and maintenance of ptn\textsuperscript{b}:** The ptn\textsuperscript{b} was isolated as a dominant enhancer of PEV after X-ray mutagenesis. It is named after Pitkin, the principal figure of Robert Ascher’s movie A Stitch in Time, who caused perplexing confusions by overdoing almost everything.

The ptn\textsuperscript{b} enhancer effect is epistatic to Su(var)3-9, a spontaneous suppressor mutation on T(2;3)ap\textsuperscript{b} in every generation the Su\textsuperscript{b} Cy ap\textsuperscript{b} red-eyed females are mated with white-eyed Su\textsuperscript{b} Cy ap\textsuperscript{b} ptn\textsuperscript{b} sibling males. For descriptions of chromosomes and marker mutations, see Lindsley and Zimm (1992) and FlyBase (2000). The ptn\textsuperscript{b} mutation, like other third chromosomal dominant female-sterile (Fs) mutations, is also maintained in a self-propagating system in which ptn\textsuperscript{b}/TM3, Sb Ser males are mated with TM3, Sb Ser/T(1;3)OR60 females (Erde\textsuperscript{ly} and Szabad 1989).

**Analysis of PEV:** The PEV modifier effect of ptn\textsuperscript{b} was studied in eight different PEV rearrangements: In(1)wm51b, In(1)wm4h, In(1)rs\textsuperscript{a}, T(1;2)N\textsuperscript{50w}; T(1;4)ap\textsuperscript{b}; T(1;4)ap\textsuperscript{a}, In(1)sc\textsuperscript{c}, and In(1)rs\textsuperscript{a}. These rearrangements differ with respect to the block of heterochromatin-inducing PEV and the region of euchromatin affected (Figure 1). In studies with u\textsuperscript{w}, w\textsuperscript{w}, rs\textsuperscript{t}, or sc\textsuperscript{e}, homozygous females were mated with w/y; ptn\textsuperscript{b}/Sb males and ptn\textsuperscript{b}/+ offspring were compared with their Sh/+ control siblings. In N\textsuperscript{50w}; w\textsuperscript{w}, Sh\textsuperscript{a}, and sc\textsuperscript{e} crosses, heterozygous FM6, y\textsuperscript{111} sc\textsuperscript{e} dm B females were used for the analysis. PEV for the genes roughest (rs, rough eyes), vertical (vt, missing dorsal thoracic bristles), scute (sc, missing scutellar bristles), and Notch (N, notched wings) was quantified by determining the proportion of flies showing expression of each mutant phenotype (penetrance). Variegation for white\textsuperscript{+} was quantified by counting the proportion of offspring with white variegated eyes or by red eye pigment measurements (Reuter and Wolff 1981). PEV for essential X chromosomal genes, whose inactivation leads to lethality in hemizygous condition, was quantified by determination of relative viability between ptn\textsuperscript{b}/+ and Sh/+ sibling males (Table 1).

**Effects of ptn\textsuperscript{b} on mini-white gene expression in P-lacW insertions:** With the exception of chromosome regions 66–68, an otherwise random selection of 62 third-chromosomal P-lacW insertions isolated by Deak et al. (1997) was tested for the effect of ptn\textsuperscript{b} on mini-white gene expression (Figure 2). w; P-lacW/TM3 females were crossed to w\textsuperscript{+}/y; ptn\textsuperscript{b}/TM3 males and eye color phenotypes compared by visual inspection of ~50 w/y; ptn\textsuperscript{b}/P-lacW and 50 w/y; P-lacW/TM3 sibling males of equivalent age. w/y; P-lacW/TM3 control males of similar age are uniform in phenotype.

**Polytene chromosome analysis:** Chromosomal distribution of SU(VAR)3-9-enhanced green fluorescent protein (EGFP) fusion protein (Schotta and Reuter 2000) was studied in polytene chromosomes of +/p[+P[GS[y\textsuperscript{b}, hs(Su(var)3-9 cDNA)]EGFP]] and ptn\textsuperscript{b}/p[+P[GS[y\textsuperscript{b}, hs(Su(var)3-9 cDNA)]EGFP]] third instar larvae after 15 min heat-shock treatment. +/p[+P[GS[y\textsuperscript{b}, hs(Su(var)3-9 cDNA)]EGFP]] larvae were received after a cross of...
ptn(GS[r])+, hs(Su(var)3-9 cDNA)EGFP]+ homozygous females with wild-type males whereas the ptn/+ pt(nGS[r], hs(Su(var)3-9 cDNA)EGFP) larvae were selected after a cross of ptnGS[r], hs(Su(var)3-9 cDNA)EGFP] homozygous females with ptn/+ T(2;3)CyO green fluorescent protein (GFP)-TM3 GFP males. The T(2;3)CyO GFP-TM3 GFP green balancer is described in Rudolph et al. (1999).

Preparation of polytene chromosomes was performed as described by Silver and Elgin (1978), with the following modifications: salivary glands of third instar larvae were dissected in PBS (130 mM NaCl, 7 mM Na2HPO4, 3 mM NaH2PO4), fixed for 10 min, and squashed in 45% acetic acid/2% formaldehyde. Chromosomes were incubated with mouse monoclonal α-GFP antibody (1:25 dilution; CLONTECH, Palo Alto, CA) at 4°C overnight, followed by incubation with a secondary FITC-conjugated goat α-mouse antibody (1:25 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 hr at 37°C. DNA was stained with propidium iodide. The preparations were mounted in Vectashield medium and examined with a confocal laser scanning microscope (LSM 510; Carl Zeiss, Thornwood, NY).

Analysis of embryonic phenotypes: Eggs of ptn/+ females do not hatch. Embryonic phenotypes were analyzed as described in Wieschaus and Nüsslein-Volhard (1986) and Jürgens et al. (1986). Genotypic determination of embryos between ptn/+ and +/+ embryos produced by ptn/+ females crossed to +/+ males was achieved by crossing y; ptn/y; TM3, Ser females to y/y; +/+ males. Head skeletons of y/y (or y/y'); ptn/y; +/+ larvae are yellow and those of the y/y (or y/y'); y/y; TM3 (ptn+)/+ +/+ larvae are dark due to y.

In crosses of ptn/+ females with ptn/+ males, three different embryonic phenotypes are observed (Figure 4). Genotypic determination of embryos was performed by PCR analysis after a cross of ptn/TM3, Ser P[w+][+4][UAS:GFP] females with ptn/P[w+] HS-hs-lacZ (65E) Sh males. The TM3, Ser P[w+][+4][UAS:GFP] and P[w+][w+][HS-hs-lacZ] (65E) chromosomes are described in (Rudolph et al. 1999) and Lu et al. (1996), respectively. Embryos were dechorionated with sodium hypochloride, transferred into PBST (PBS plus 0.2% Tween 20), briefly fixed in methanol followed by washing four times in PBST, rehydrated for 1.5 hr in PBST, 4% 6-diamidino-2-phenylindole (DAPI) stained, briefly washed in PBST, and inspected under a UV microscope. Very early arrested, late cleavage arrested, and gastrulated embryos were individually collected. From each type, five embryos were collected and DNA preparation was performed according to Gloor and Engels (1992). Two primer pairs were used for PCR detection of wild-type (wt)GFP and lacZ, respectively (wtGFP forward 5’-AGTGAGAGGGGTGAAGTGATG and wtGFP reverse 5’-AAGGGCGAGTTGGTGACAGC; lacZ forward 5’-TGA CCTGAGGCGATTTTAC and lacZ reverse 5’-GGAGGCGACATTCACTTAAGC). The size of the expected fragments is 534 bp for wtGFP and 505 bp for lacZ. In TM3, Ser P[w+][+4][UAS:GFP]/ptn (paternal) embryos the wtGFP-specific fragment is amplified whereas from P[w+][HS-hs-lacZ] (65E) Sh/ptn (maternal) embryos the lacZ-specific fragment is received. Amplification of both the GFP- and lacZ-specific fragments identifies the TM3, Ser P[w+][+4][UAS:GFP]/P[w+][HS-hs-lacZ] (65E) Sh = ptn+/ptn− embryos.

Early cleavage defects in embryos derived from ptn/+ females were analyzed in 0- to 1-h-old DAPI-stained embryos with a Zeiss fluorescent microscope, or, after staining with propidium iodide, immunostaining of tubulin (YLI/2 rat monoclonal anti-tubulin, Serva), and of the nuclear lamina (T47 anti-lamin monoclonal antibody; Amersham, Arlington Heights, IL; Paddv et al. 1996), with the confocal laser scanning microscope. The specimens were incubated in the primary antibodies overnight (4°C). The secondary antibodies (Jackson Laboratories, Avondale, PA) were applied for 2 hr at room temperature. Specimens were mounted in 85% glycerol containing 2.5% n-propyl gallate. Optical sections were generated in a Zeiss 410 or Zeiss LSM510 confocal laser scanning microscope.

Mapping of the pithin locus: For crossover mapping of ptn we made use of the polygenic modifiers present in the ru ca ca inbred strain (Erdei ly and Szabad 1989). Some of the w/+; ru ca/ptn females gave rise to a few offspring. Recombinant offspring were identified after a backcross to w/ru; y ru ca ru ca ca males. According to the genetic map position determined, transheterozygotes of ptn with different Dp(3;3)S2a chromosomes (Craymer 1984) covering region 64C-71D were studied for rescue of dominant female sterility (Figure 6). Further refinement of the map position has been achieved by overlapping deficiencies within region 67-68 and Pelelement-induced ptn mutations by testing for the no egg phenotype, a diagnostic feature for ptn+/ptn− transheterozygous females (Figure 7).

Reversion of ptn and Pelelement-induced pithin mutations: ptn was a gain-of-function mutation whose female sterility and dominant PEV enhancement phenotypes are reversible using X rays and Pelelement hybrid dysgenesis, respectively. X-ray-induced revertants were isolated after irradiating ptn/TM3, Sh Ser males with 4000 R of X rays. These males were crossed to TM3, Ser/TM3 females and revertants were selected as fertile ptnHS-lacZ/ TM3, Ser or ptnHS-lacZ/TM3 females where rX denotes the X-ray-induced revertant chromosomes (TM3, Sh Ser/TM3, Ser and TM3, Sh Ser/TM3 are lethal). For isolation of Pelelement hybrid dysgenesis-induced revertants, P cytotype In(3LR)CxD/ TM3, Ser females were crossed to ptn/TM3, Sh Ser males. The F1, ptn/ CxD males were backcrossed to M cytotype w/+; TM3, Sh Ser/Pr Dr females. The w/+; Y; TM3, Sh Ser/ptn− male offspring were then crossed to w/+; In(2L)Cy+In(2R)Cy, Cy cvr spf/T(2;3)hapb, apb Su(var)2-115/S6 females. T(2;3)hapb, apb Su(var)2-115 male offspring were inspected for reversion of the strong enhancer effect of ptn (red-pigmented instead of white eyes).

After screening of the P-lacW insertion collection of Deak et al. (1997) for recessive ptn alleles, the two P-lacWinduced ptnw890 and ptnw890/2 mutations were identified on the basis of their no egg phenotype over ptn. The P-lacW element carries the w marker gene and excisions of the element can be selected by loss of w function. Revertant analysis was performed with both ptnw890 and ptnw890/2 by selection of w− offspring in a cross of w+/w, TM3/TM3 females to w/ptn−/ptn− or ptnw890/ptn−/TM3, ΔZ-2 males. The TM3, rY880 P[cy]ΔZ-3 (99B) balancer chromosome contains a stable source of transposable which is efficient in P-element remobilization (Lu et al. 1993).

Ovarian phenotypes: Homozygous ptn+/ptn− females were recovered at a very low frequency in crosses of ptn+/ In(3LR)CxD females to ptn+/TM3 males. The ptn+/CxD females for this cross were generated by a series of crosses with a ru ca ca multiply marked stock, which was shown to contain modifiers that reduce the penetrance of several dominant female-sterile mutations (Erdei ly and Szabad 1989). The ptn gene is uncovered by Df(3L)AC1 (see RESULTS). The two P-lacW inserts ptnw890 and ptnw890/2 (Figure 7) represent recessive ptn mutations. The ptnw890/Df ptn− and ptn/Df ptn− females were generated by crossing Df[3L]AC1/TM3 females with ptn+/ TM3, ptnw890/1/TM3 or ptnw890/2/TM3 males, respectively. Ovaries of 7- to 10-day-old females were dissected, fixed, and analyzed as described previously (Theuerkauf and Hawley 1992). Staging of egg primordia is according to Swedlow et al. (1995).
treatment produces +/+ germ-line cells in ~10% of the irradiated females. To test whether ptnD-free germ-line cells give rise to offspring, irradiated females were crossed to w^+ /Y; w^h th/+ h th males and monitored for offspring production over a period of 15 days.

Germ-line chimeras: Three types of germ-line chimeras were constructed through transplantation of blastocysts (ILMENSEE 1973; VAN DEUSEN 1977). (1) Pole cells of donor embryos produced by a cross of w^+/+ w^h; vth/THM3 females were transplanted into host embryos that originated from a cross of wild-type females with ado^+/+ Y males. ado^h is a dominant female-sterile mutation that alters function of the germ line without affecting the soma (BUSSON et al. 1983; KOMITOPOLOU et al. 1983). ado^+/+ females’ offspring, which might have received ptnd^+/+ or w^h th or w^h th/THM3 pole cells, were mated with w^h/Y; +/+ males. (2) Pole cells from embryos produced by a cross of fs(1)k10/CLB females with fs(1)k10 w^h/Y males were transplanted into host embryos produced by a cross of w^+/+ w^+/+; vth th/+ h th females with w^+/+ Y; ptnd^+/+ TM3 males. Ecloding females were mated with fs(1)k10 w^h/Y females. fs(1)k10 is an egg-shaped marker mutation in which the mutant phenotype depends on the genotype of the germ-line cells (WIESCHAU ET AL. 1978). (3) Pole cells from donor embryos produced by y y w^h; ptnd^+/+ /TM6b, Tb females were crossed to y w^h; ptnd^+/+ /TM3, Sh Ser array males were transplanted into host embryos derived from a cross of wild-type females with ado^+/+ Y males. Ecloding females were mated with y w^h/Y males.

Follicle cell mosaic analysis: To determine the effect of the ptnd gene on follicle cell development we generated homozygous ptnd^+/+ follicle cell clones by X-ray-induced mitotic recombination in ptnd^+/+(F3)Apc females. F3(Apc) is a dominant female-sterile mutation that only disrupts follicle cell function without interfering with function of germ-line cells (ERDEHLYI and SZABAD 1989). For induction of ptnd homozygous follicle cells, F3(Apc)/ptnd^+/+ heterozygous females were irradiated with 1500 R X-rays (150 kV, 0.5-mm Al filter, 1000 R/ min) as early third instar larvae or adults and afterward tested for offspring production. Whether ptnd homozygous follicle cells support egg development can be determined by comparing offspring production frequencies between ptnd^+/+ /F3(Apc) and +/F3(Apc) (control) females.

RESULTS

ptnd is the strongest known enhancer mutation of PEV: The ptnd mutation was isolated after X-ray mutagenesis of wild-type chromosomes on the basis of its very strong PEV enhancer effect on white variegation in w^h. The mutation was formerly described as E-var(3)206 (REUTER ET AL. 1985). Normally only variegation of the white gene is observed in w^h flies and enhancement of PEV is evident by the more frequent inactivation of the white gene and by inactivation of genes located more distally from the breakpoint. w^+/+ Y; ptnd^+/+ males express a complete w and rst eye mutant phenotype, show variegation for vt, and inactivation of essential genes more distally located from the breakpoint is indicated by their reduced viability (Table 1, Figure 1). In w^+/+; ptnd^+/+ females Notch variegation becomes visible. Reversion of variegating rearrangements is accompa-

strong enhancer capability of ptnd, we tested in an earlier study its effect on 52 w^h revertant chromosomes of w^+/+ (REUTER ET AL. 1985). ptnd induced white variegation in 50 out of the 52 w^h revertant chromosomes. Enhancement of PEV by ptnd in w^h is also visible in Malpighian tubules. Regularly, in w^h larvae only about one to two white cells in Malpighian tubules are found whereas large white sectors are seen in w^h; ptnd^+/+ larvae (data not shown).

Strong enhancement of PEV by ptnd is also observed for all of the other analyzed rearrangements (Figure 1, Table 1). The different rearrangements juxtapose various euchromatic regions to different blocks of heterochromatin (Figure 1). rst^− flies usually show only rst variegation and infrequently they exhibit variegation of vt. In rst^−/Y males, ptnd^+ induces complete inactivation of rst, strong variegation of vt, and the significant reduction of viability of these males indicates inactivation of more distally located essential genes (Figure 1, Table 1). In rst^−/w; ptnd^+/+ females w and N variegation appears. The enhancer effect of ptnd on white variegation in w^h was quantified by red eye pigment measurements. w^h/Y; ptnd^+/+ males show strong w mottling and variegate for rst and vt, which is never found in control genotypes (Table 1). T(1;2)N^264^20 variegates weakly for N, but not for wt. In contrast, all T(1;2)N^264^20/ w; ptnd^+/+ females show w variegation and N variegation is strongly enhanced (Table 1, row 4). In T(1;4)w^h^2532 the ptnd mutation results in a strong N mutant phenotype in all females and w variegation is strongly elevated as shown by red eye pigment measurement (Table 1, row 5). Significant enhancement of w variegation is found in T(1;2)N^+/+ w; ptnd^+/+ females (Table 1, row 6). ptnd also enhances variegation of the sc gene in sc^− as indicated by reduction of the mean number of scutellar bristles (Table 1, row 7, column 6). At low temperature of development inactivation of essential genes distal to sc in sc^− is indicated by reduced viability of sc^−/Y; ptnd^+/+ males (Table 1, row 7, column 5). There is no significant inactivation of essential genes at the proximal breakpoint of sc^− (BAKER 1971). ptnd significantly enhances inactivation of essential genes at this breakpoint, as indicated by the marked reduction of sc^−/Y male viability (Table 1, row 8). In all the PEV rearrangements studied ptnd strongly promotes spreading of heterochromatin-induced gene inactivation along euchromatic regions and loci more distant from the breakpoint become subjected to gene silencing (Figure 1).

ptnd represses mini-white gene expression at euchromatic insertion sites and causes ectopic distribution of heterochromatin protein SU(VAR)3-9: We have analyzed the effect of ptnd on mini-white gene expression in 61 P-lacW elements inserted at random sites within the third chromosome (Figure 2). In 23 of the 61 inserts studied (~38%), mini-white gene expression was strongly reduced in ptnd/P-lacW males. In only one case (P-lacW 1018/1), a variegated expression of the mini-white gene
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<th>Rearrangement</th>
<th>Genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sex chromosomes</th>
<th>Autosomes</th>
<th>No. of flies</th>
<th>Viability (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>White variegation in % of eyes (red eye pigments)</th>
<th>Roughest variegation (%) of flies</th>
<th>Vertical variegation (%) of flies</th>
<th>Notch variegation (%) of flies</th>
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</tr>
<tr>
<td>T(1;2)wm&lt;sup&gt;258&lt;/sup&gt;-21</td>
<td>wm&lt;sup&gt;258&lt;/sup&gt;-21 /w</td>
<td>+ / TM3</td>
<td>119</td>
<td>100 (104.1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>ptn&lt;sup&gt;b&lt;/sup&gt;/+</td>
<td>149</td>
<td>100 (31.3)</td>
<td>—</td>
<td>—</td>
<td>0</td>
<td>30.9</td>
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<tr>
<td>T(1;2)Na8</td>
<td>Na8 / w</td>
<td>+ / TM3</td>
<td>135</td>
<td>100 (7.7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td>ptn&lt;sup&gt;b&lt;/sup&gt;/+</td>
<td>139</td>
<td>100 (3.6)</td>
<td>—</td>
<td>—</td>
<td>Notch&lt;sup&gt;+&lt;/sup&gt;</td>
<td>—</td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Homozygous w<sup>*</sup> females were crossed to w<sup>*</sup>/Y; ptn<sup>b</sup>/TM3, Sb males. Otherwise the homozygous (rst<sup>e</sup>, w<sup>51</sup>, sc<sup>e</sup>) or heterozygous (N<sup>264</sup>-10, wm<sup>258</sup>-21, N<sup>8</sup>, sc<sup>S1</sup>) females were crossed to w/Y; ptn<sup>b</sup>/TM3, Sb males. PEV in + / TM3, Sb control and ptn<sup>b</sup>/ + sibling genotypes was compared.

<sup>b</sup> Percentage of control. Unless otherwise indicated, the fly cultures developed at 25°.

<sup>c</sup> = phenotypically not detectable inactivation of the gene (due to recessive phenotype or lethality). Red eye pigments in percentage of wild-type Canton-S flies are shown.

<sup>d</sup> Mean number of scutellar bristles.
Figure 1.—*ptn*D increases the spreading of inactivation in PEV rearrangements. (A) Schematic representation of eight different chromosomal rearrangements that juxtapose different blocks of constitutive heterochromatin (thick bars) to different segments of euchromatin (thin lines) and induce PEV for the genes shown. Arrows illustrate the direction of the heterochromatin-induced gene silencing. In *T(1;4)*Na8 and *T(1;4)*wm258-21 the dotted blue lines indicate a significantly lower frequency of variegation in 1/1 as compared to *ptn*D/1 (red lines). (B) Photographic representation of the enhancer effect of *ptn*D on *wm4*. Variegated expression of the *white* gene, associated with the *wm4h* inversion, leads to the formation of red-white spotted eyes. The strong enhancer effect of *ptn*D causes complete inactivation of the *white* and *roughest* genes.

was observed. These results suggest an effect of *ptn*D on both heterochromatic and euchromatic regions. In almost all of the *P-lacW* inserts tested, *mini-white* gene expression is already repressed in the control and *ptn*D might enhance stable position effects at certain euchromatic regions. Alternatively, the effects of *ptn*D on euchromatic *mini-white* gene insertions could be caused by a mechanism related to heterochromatin-induced gene silencing in PEV. To test this hypothesis, we studied chromosomal distribution of heterochromatin protein SU(VAR)3-9 in salivary gland giant chromosomes of wild-type and *ptn*D/+ larvae (Figure 3). In wild type the SU(VAR)3-9EGFP protein is almost exclusively associated with chromocenter heterochromatin and the fourth chromosome (Schotta and Reuter 2000; Figure 3A). In contrast to this, >100 ectopic SU(VAR)3-9EGFP binding sites along all chromosome arms are detected in salivary gland chromosomes of *ptn*D/+ larvae (Figure 3B). An identical ectopic distribution of SU(VAR)3-9EGFP is also found in unfixed salivary gland nuclei (Figure 3D). Most of the ectopic sites correlate with heavily stained bands. Furthermore, all of the affected *mini-white* gene insertions are located at chromosomal sites where ectopic SU(VAR)3-9EGFP is detected.
**Figure 2.**—$ptn^D$ reduces the expression of the mini-white gene in $P$-lacW inserts. Expression of mini-white gene is significantly reduced in the inserts shown above the schematic cytological map. Examples are shown for three different lines (495/8, 40/21, and 501/15; control 700/16 with no effect). $ptn^D$ has no effects on mini-white gene expression in the inserts shown below the map.
Our results indicate that ptnD-induced repression of euchromatic mini-white insertions is caused by ectopic binding of heterochromatin protein complexes. ptnD is the first modifier of PEV mutations that is shown to induce ectopic distribution of a heterochromatin-associated protein.

ptnD induces dominant female sterility through maternal-effect and zygotic lethality: ptnD/+ females exhibit wild-type fecundity and all of their eggs are fertilized (data not shown). However, larvae do not hatch from the eggs. When ptnD/+ females are mated with wild-type (+/+) males, two types of embryos can be differentiated after DAPI staining (Figure 4). In ~50%, development is arrested shortly after initiation of cleavage divisions. Developmental arrest of these embryos was studied after 2-hr aging of eggs collected for a 1-hr period. DAPI staining revealed that most did not initiate cleavage or were arrested around cleavage cycle 1–3 (88.5%; 54/61) and a maximum of six small nuclei that appear abnormal in chromatin structure were visible (Figure 6). Only 11.5% (7/61) reached cleavage cycles 4–10. The other 50% of the embryos developed to the larval stage of differentiation. They had defective cephalopharyngeal head skeletons (Figure 5) and did not hatch. To determine the genotype of the two types of embryos, we crossed y/y; ptnD/Y TM3 females with y/Y; +/+ males (all TM3 chromosomes are ptnD; cf. MATERIALS AND METHODS). All embryos with an abnormal head skeleton were yellow+ in phenotype and hence they inherited y+/TM3 from the ptnD/Y mother. Therefore, lethality of the +/+ embryos derived from ptnD/+ mothers must be due to maternally provided ptnD gene product. Zygotes in which development is arrested shortly after fertilization are thus ptnD/+ and received the ptnD allele from their ptnD/+ mothers. Therefore, the presence and early expression of the ptnD...
Figure 4.—Embryonic phenotypes associated with \( ptn^D \). \( ptn^D/1 \) females crossed with \(+/1\) wild-type males produce two types of embryos at a 1:1 ratio: (A) The chromatin appears highly condensed after one to three cleavage divisions in the \( ptn^D \) (maternal)/\(+\) embryos leading to termination of embryogenesis. (B) The \(+/+\) embryos reach the blastoderm stage and stop development at the end of embryogenesis (cf. Figure 5). (C) A third phenotype is observed in \(~25\%\) of embryos from the matings of \( ptn^D/1 \) females and \( 1/1 \) males. Chromatin of late cleavage nuclei becomes highly condensed in these \( +/ptn^D \) (paternal) embryos. Determination of embryonic genotypes was performed after a cross of \( ptn^D/TM3\) GFP females with \( ptn^D/lacZ\) Sb males by PCR analysis. Amplification of the lacZ- or GFP-specific DNA fragments allowed differentiation between \( ptn^D \) (maternal)/\(+\) and \(+/ptn^D\) (paternal) embryos. All embryos were DAPI stained. (Magnification \( \times100\).)

Three types of embryos develop from \( ptn^D/1 \) females crossed to \( ptn^D/+\) males (Figure 4). Cleavage divisions did not initiate in \(~50\%\) of the zygotes, and \(~25\%\) of the embryos had abnormal head skeleton development \((+/+\) zygotes\). Each of the remaining \(25\%\) of the embryos possessed several hundred brightly DAPI-fluorescent small nuclei that are irregularly distributed (Figures 4 and 6). In these embryos, cleavage initiates but the nuclei never populate the entire cortex and no blastoderm is formed. Genotypes of the different classes of embryos were determined by PCR after a cross of \( ptn^D/TM3\), GFP females with \( ptn^D/lacZ\) Sb males (cf. MATERIALS AND METHODS and Figure 4). PCR amplification of a GFP or lacZ-specific DNA fragment allowed differentiation between \( ptn^D \) (maternal)/\(+\) \((TM3,\) GFP\) and \( ptn^D \) (paternal)/\(+\) \((lacZ,\) Sb\) embryos, respectively (Figure 4).

As shown by PCR analysis, the genotype of embryos that die after cleavage is \(+\) (maternal)/\( ptn^D \) (paternal) (Figure 4). Cleavage nuclei of these embryos appear
strongly condensed and chromatin is frequently fragmented (Figures 4 and 6). This might be due to additive/synergistic effects of the maternally and paternally derived mutant ptn\textsuperscript{D} gene products. In accordance with this suggestion, all embryos produced by +/+ females that were crossed to ptn\textsuperscript{D}+/ males develop normally.

The pithin gene is located in the 67C3-5 cytological region: A few offspring develop from 1% of the ptn\textsuperscript{D}/ rucaua females (cf. MATERIALS AND METHODS). The recombinant offspring from a test cross between w\textsuperscript{act}/w\textsuperscript{act}; ptn\textsuperscript{D} ru cu ca females and w\textsuperscript{act}/Y; ru cu ca /ru cu ca males allowed crossover mapping of the ptn locus. All recombinant chromosomes were tested for dominant female sterility and enhancement of PEV. ptn\textsuperscript{D} is located within the interval delineated by the hairy (h) and the thread (th) marker mutations (Figure 6). The 66 recombinants isolated (52 h ptn\textsuperscript{D} + and 14 + ptn\textsuperscript{D} th) divided the h-th interval at a 52/14 ratio. Taking the position of the h and th loci as reference points (26.5 and 43.2 cM, respectively; LINDSLEY and ZIMM 1992) the ptn is located at 39 cM. The dominant female sterility and the dominant PEV enhancer effect of ptn\textsuperscript{D} were not separated by any of the recombinants, suggesting that the two phenotypes stem from the same mutation.

Genetic map position 39 cM corresponds to the 66D(h)–72B(th) cytological region (LINDSLEY and ZIMM 1992; Figure 7). We combined ptn\textsuperscript{D} with a series of Dp(3;3)S2a chromosomes (CRAYMER 1984) and tested for rescue of ptn\textsuperscript{D} mutant phenotypes (Figure 7). Larvae hatched and developed to adulthood from 38 and 18% of the eggs deposited by ptn\textsuperscript{D}/Dp(3;3)S2a2 and ptn\textsuperscript{D}/Dp(3;3)S2a8 females, respectively. None of the other duplications showed a rescue effect on ptn\textsuperscript{D}-associated dominant female sterility. This partial rescue of dominant female sterility in ptn\textsuperscript{D}+/Dp ptn\textsuperscript{-} females shows that ptn\textsuperscript{D} resides within the 66D; 67D–E chromosome segment and indicates an antimorphic nature of the ptn\textsuperscript{D} mutation. This result also implies an involvement of both the normal and the ptn\textsuperscript{D}-encoded mutant gene products in the same process. Comparison of white motting between w\textsuperscript{act}/Y; ptn\textsuperscript{D}+/ and w\textsuperscript{act}/Y; ptn\textsuperscript{D}/+Dp ptn\textsuperscript{-} males did not reveal a significant effect of the duplications on the dominant PEV enhancer effect of ptn\textsuperscript{D}.

Complementation analysis with Df(3L) chromosomes indicated that Df(3L)AC1 uncovers the ptn locus. ptn\textsuperscript{D}/ Df(3L)AC1 (= ptn\textsuperscript{D}/Df ptn-) females do not deposit eggs, suggesting a location of the ptn gene within 67B–67D. All the egg primordia in ptn\textsuperscript{D}/Df(3L)AC1 degenerate prior to vitellogenesis and excess chromatin condensation is apparent in nurse cell nuclei (Figure 8C). A comparable phenotype is found in egg primordia of ptn\textsuperscript{D}/ptn\textsuperscript{D} females (Figure 8).

We have tested all of the P-lacW insertions in the 67B–67D region from the DRAG et al. (1997) collection of third chromosomal P-element-induced mutations for alleleism with ptn\textsuperscript{D}. Two of the P-induced mutations (ptn\textsuperscript{D}-9003 and ptn\textsuperscript{D}-9004) failed to complement ptn\textsuperscript{D}. The ptn\textsuperscript{D}/ptn\textsuperscript{D}--insert females do not produce vitellogenic egg primordia, and as in ptn\textsuperscript{D}/ptn\textsuperscript{D} and ptn\textsuperscript{D}/Df ptn- females, chromatin of nurse cell nuclei also appears to be more condensed in ptn\textsuperscript{D}-9003/ptn\textsuperscript{D} and ptn\textsuperscript{D}-9004/ptn\textsuperscript{D}; egg primordia (Figure 8, E and F). In these egg primordia propidium iodide does not stain nurse cell cytoplasm, indicating an unusually low amount of RNA (Figure 8, B, C, and F). It remains to be determined whether this indicates repression of gene activity in nurse cell nuclei. Neither of the ptn\textsuperscript{D}-insert alleles causes dominant or recessive enhancement of PEV. In situ hybridization analysis revealed that the two P-element insertions are located in region 67C3-5 (DEAK et al. 1997), narrowing the cytogenetic location of the ptn locus further.

Reversion analysis of ptn\textsuperscript{D}: Both X-ray- and P-induced revertants of ptn\textsuperscript{D} were isolated in this study. The X-ray revertants were isolated on the basis of loss of dominant female sterility whereas the P revertants were isolated by reversion of PEV enhancement. The two kinds of revertants are designated as ptn\textsuperscript{D}- and ptn\textsuperscript{D}+, respectively. From 4728 X-ray-treated ptn\textsuperscript{D} chromosomes, 11 carried a ptn\textsuperscript{D}- allele. None of the ptn\textsuperscript{D}- chromosomes
ptnD affects structure of early cleavage nuclei. Wild-type embryos (A, C, E, and G), ptnD(maternal)/+ (B and D) and +/ptnD(paternal) embryos (F and H; cf. Figure 4) are shown. In the optical sections, DNA is stained with propidium iodide and labeled red. Nuclear lamina (C, D, G, and H) and tubulin (A and B) appear green. ptnD(maternal)/+ embryos show chromatin fragmentation and chromatin bridges during early cleavage (B) and the irregularly sized interphase nuclei appear condensed (D). The +/ptnD(paternal) embryos (F and H) show clumps of strongly condensed and fragmented chromatin. Nuclear lamina in wild-type nuclei surrounds chromatin (C and G) whereas it appears disintegrated and is no longer clearly separated from chromatin (yellow staining) in ptnD/nuclei (D and H). Note the different levels of magnification as labeled in the bars.

enhances PEV in heterozygous condition, showing that ptnD-associated dominant female sterility and the enhancement of PEV revert concomitantly; this suggests that the two phenotypes stem from the same mutation. On the other hand, two ptnD paternal alleles were recovered from among 20,000 chromosomes tested for a loss of the PEV enhancer effects of ptnD. The ptnD paternal alleles do not induce dominant female sterility. The fact that ptnD is revertible by these two mutagenic agents supports our contention about its gain-of-function nature. Altogether 9 of the isolated revertant chromosomes are homozygous viable and fertile and only 4 are recessive lethal.

Revertant analysis of the insertional ptnD lacW alleles: We found that for both ptnD lacW alleles, precise excis-
Figure 7.—Cytogenetic mapping of the ptn gene in region 67C. Breakpoints of duplications [Dp(3;3)S2a] and deficiencies [Df(3L)] used are indicated at the right. The two P-lacW insertions at region 67C3-5 represent recessive ptn mutations and showed that the ptn locus resides in the 67C3-5 cytological region.

ion results in reversion of the ptn mutant phenotype. ptnD0 excisions were selected by loss of the mini-white marker gene. In the analysis of ptnP99/2 a total of 11 revertant chromosomes were isolated. A short flanking genomic region was cloned by inverse PCR. Consecutive analysis of revertant chromosomes revealed a second defective P-element ~500 bp next to the P-lacW element (Figure 9). PCR analysis using a primer pair complementary to sequences from the genomic region flanking P-lacW and the 3′ Prepeat yielded two different-sized fragments for the revertant chromosomes. In the ptnP99/2 revertants 2, 3, 4, 5, 8, 10, and 11, a fragment of ~0.8 kb was generated, indicating excision of the P-lacW element. In these chromosomes the primers amplified the region between the flanking genomic sequence and whether the ptnD-associated dominant female sterility is the consequence of an altered function of germ-line or the 39 P-repeat of the adjacent defective element. PCR analysis of the ptnP99/2 revertants 1, 6, 7, and 9 yielded a 300-bp fragment in all cases, indicating the presence of the 3′ Prepeat of the P-lacW element (Figure 9). These revertants therefore contain internal deletions within P-lacW affecting the mini-white marker gene. All revertants that represent excisions of P-lacW complement ptnD0, whereas those that represent internal deletions within P-lacW do not complement ptnD and show an ovarian phenotype identical to ptnD/Dfptn females. In the analysis of the ptnP99/4 allele, a total of 69 w- revertants were isolated. Of these, 22 are recessive female sterile, 41 are homozygous female fertile, and 6 are recessive lethal. With respect to the sterile lines, the homozygous females either did not lay any eggs or deposited only a few eggs that did not develop further. We propose that all of the ptn alleles that produce no P-lacW and the 39 P-repeat yielded two different-sized fragments for the revertant chromosomes. In the ptnP893/2 eggs are amorphs for the ptn gene function. Germ-line mosaics and chimeras show the gain-of-function nature of ptnD as well as an essential function of the ptn gene in female germ-line cells: To study whether the ptnD-associated dominant female sterility is the consequence of an altered function of germ-line or somatic cells we analyzed germ-line mosaics. First, mitotic recombination was induced in w+/w+, ptnD/w+ th and w+/w+; ptnD/se ss e ro adult females for the generation of ptnD-free +/+ germ-line cells. If ptnD alters the function of germ-line cells, offspring are expected to derive from the +/+ germ-line cells generated by
mitotic recombination. Only 2 (0.1%) of the 2029 \( ptn^D/\text{ve} h \text{th} \) and \( ptn^D/\text{se} ss e \text{ ro} \) irradiated adult females gave rise to one offspring each. Based on the frequency of mitotic recombination, \(~200\) of the 2029 \( ptn^D/+ \) females were expected to carry \( ptn^D\)-free germ-line clones (Erdélyi and Szabad 1989). This striking difference between the expected and observed frequencies may be explained by suggesting either that the \( ptn^D\)-induced sterility originates from altered function of the somatic cells or that \( ptn^D \) alters function of the female germ line and an extensive perdurance of the \( ptn \) mutant product prevents development of +/+ germ-line clones. The latter explanation supports the contention that the \( ptn^D \) allele has gain-of-function properties. Perdurance of the mutant gene product was already described for a large fraction of dominant female-sterile mutations isolated by Erdélyi and Szabad (1989) and Szabad et al. (1989). To distinguish between a possible perdurance effect or a nongerm-line autonomous function of \( ptn^D \), we constructed two types of germ-line chimeras through pole cell transplantation. First, \( ptn^D/+ \) pole cells were transplanted into \( ovd^D/+ \) host embryos. Six chimeras carried \( ptn^D/+ \) germ-line cells (Table 2A). Typical dead embryos with abnormal head skeleton developed in \(~50\%) of their eggs. The other 50% of the eggs showed very early arrest typical for the \( ptn^D/+ \) mutant phenotype. Therefore, \( ptn^D\)-related female sterility is germ-line dependent. This is further supported by the results of an experiment where nonmutant pole cells were implanted into \( ptn^D/+ \) host embryos (Table 2B). The three chimeras with nonmutant germ-line cells and \( ptn^D/+ \) soma each gave rise to offspring originating from the implanted germ-line cells in addition to their eggs from which no larvae hatched. To further characterize the function of the \( ptn \) gene in female germ-line and follicle cells, we constructed germ-line chimeras in which normal follicle cells surround \( ptn/\text{ptn} \) germ-line cells (Table 2C). In these studies, the Pelement-induced recessive female-sterile \( ptn^890^4 \) and \( ptn^895^2 \) mutations were used (Materials and Methods). Each of the five germ-line chimeras deposited only very few eggs throughout the 2-wk test period. There was no indication of embryonic development, showing that the normal function of the \( ptn \) gene is required in the germ-line components of the egg primordia.

The possible role of the \( ptn \) gene in follicle cells was studied following induction of \( ptn/\text{ptn} \) homozygous follicle cell clones in \( ptn/\text{Fs}(3)Apc \) females. After X-ray-induced mitotic recombination in larvae and adults, both the \( ptn/\text{Apc} \) females and the +/\text{Apc} control females
produced offspring at similar rates (Table 3), showing that the ptn gene is not required for normal follicle cell function. Our analysis of the germ-line and follicle cell mosaics shows that the ptnO-induced sterility is due to an altered function of female germ-line cells. Our results also provide strong evidence for our contention of a gain-of-function nature for the ptnO allele, its expression in the female germ line, and induction of embryonic lethality by the maternally contributed ptnO-encoded mutant gene product.

**DISCUSSION**

ptnO represents the first gain-of-function type enhancer of PEV described in Drosophila. Almost all modifier mutations of PEV represent loss-of-function type alleles and only recently gain-of-function type suppressor mutations were identified (Clegg et al. 1998; Mottus et al. 2000). In addition to its strong dominant enhancer effect on PEV, ptnO causes dominant female sterility. It affects female germ-line development, early embryogenesis, enhances PEV, induces ectopic white gene silencing in somatic cells, and causes ectopic association of heterochromatin protein Su(VAR)3-9 with numerous euchromatic sites. Furthermore, genetic analysis shows that ptn represents a novel PEV modifier gene. The known PEV modifier genes are characterized by a dosage-dependent effect on PEV (Henikoff 1979; Reuter and Szidonya 1983; Tartof et al. 1984; Wustmann et al. 1989). They manifest a haplo-dependent effect either as suppressors or enhancers of PEV; i.e., a 50% reduction of the wild-type gene product results in a dominant phenotype. In contrast, neither a deletion of the ptn gene nor any of the hypomorphic and loss-of-function ptn alleles have dominant effects on PEV. Several of the classical PEV modifier genes express triplo-dependent effects; these fall into two groups, haplo-suppressor with a triplo-dependent enhancer effect and haplo-enhancers with a triplo-dependent suppressor effect (Wustmann et al. 1989). Three of the former genes have been molecularly characterized and all were shown to encode heterochromatin-associated proteins. These genes are Su(var)2-5, which encodes HP1 (James and Elgin 1986; Eissenberg et al. 1992), Su(var)3-7, which codes for a protein with multiple zinc fingers (Reuter et al. 1990; Cleârd et al. 1997), and Su(var)3-9, a gene that encodes a protein containing the chromo and SET domains, two evolutionarily conserved motifs also found in many other chromatin proteins (Tschiersch et al. 1994; Jenuwein et al. 1998; Schotta and Reuter 2000). Overproduction of these proteins results in strong enhancement of PEV. Duplications covering the ptn gene do not show any effect on PEV. Therefore, the ptn gene does not belong to any of the different groups of PEV modifier loci described to date and the various effects of ptnO are clearly due to its gain-of-function nature. This is supported by the following three findings:

**Figure 9.—Reversion analysis of ptnP893/2.** (Top) Organization of the genomic region and (bottom) PCR analysis of revertant chromosomes. Primer 893/2genom: 5'-CAA CGC TCT CTT AAC CTG TC-3'. In revertants due to complete excisions of PlacW an ~800-bp fragment is amplified between 893/2genom and the 3' P-element sequences of a second defective element.
TABLE 2
Features of ptnp/+ and ptn/ptn germ-line chimeras

<table>
<thead>
<tr>
<th>Genotype of embryos</th>
<th>Types and nos. of germ-line chimeras</th>
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</thead>
<tbody>
<tr>
<td>Donor</td>
<td>Host</td>
</tr>
<tr>
<td>A. ptnp/+ germ-line cells surrounded by normal soma</td>
<td></td>
</tr>
<tr>
<td>ptnp/w th TM3</td>
<td>avdp/+</td>
</tr>
<tr>
<td>ve th/ TM3</td>
<td>avdp/+</td>
</tr>
<tr>
<td>B. Normal germ-line cells surrounded by ptnp + soma*</td>
<td></td>
</tr>
<tr>
<td>+/-</td>
<td>ptnp/w th TM3</td>
</tr>
<tr>
<td>+/-</td>
<td>ve th/ TM3</td>
</tr>
<tr>
<td>C. ptn/ptn germ-line cells surrounded by normal soma</td>
<td></td>
</tr>
<tr>
<td>ptnp/w th TM3</td>
<td>avdp/+</td>
</tr>
<tr>
<td>TM3/TM6</td>
<td>avdp/+</td>
</tr>
<tr>
<td>ptnp/w th TM3</td>
<td>avdp/+</td>
</tr>
</tbody>
</table>

In A the donor and in B the host embryos derived from a cross between w /w; ve th/ve th females and w/Y; ptnp/male. Pole cells of the donor embryos in A and C were transplanted into host embryos that derived from a cross of wild-type (Canton-S) females and avdp/Y males. In C donor embryos derived from a cross of y w/y w; ptnp/TM6b, Th females with y w/Y; ptnp/TM6b, Sb Ser males. Pole cells of the donor embryos were transplanted into host embryos that derived from a cross of wild-type females with avdp/Y males. Eclosing females were mated with y w/Y males.

* The donor embryos in B derived from a cross of fs(1)K10 w/ClB females with fs(1)K10 w/Y males. Their genotypes are fs(1)K10 w/ClB; fs(1)K10 w and ClB; fs(1)K10 w.

1 All embryos received K10 w/ClB pole cells from which progeny developed. Eggs of the ptnp/ve th host females did not hatch.

2 Besides their own eggs six of the ve th/ TM3 host females deposited K10 eggs and three produced offspring from K10 w/ClB pole cells (besides their own progeny). Chimera with ptnp/w th TM3 germ-line cells did not lay eggs. Their egg primordia were similar to those that develop in ptnp/Df(3L)A17 females.

TABLE 3
The effect of ptnp on homozygous mutant follicle cell clones

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Genotype of females</th>
<th>Females tested</th>
<th>Mosaics</th>
<th>Frequency of mosaicism (%)</th>
<th>Offspring</th>
<th>Offspring production</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>+/Apc</td>
<td>226</td>
<td>1</td>
<td>0.4</td>
<td>1</td>
<td>0.12 × 10⁻⁵</td>
</tr>
<tr>
<td>None</td>
<td>ptnp/Fs(3)Apc</td>
<td>218</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
<td>0.14 × 10⁻⁵</td>
</tr>
<tr>
<td>Larvae</td>
<td>+/Apc</td>
<td>359</td>
<td>30</td>
<td>8.3</td>
<td>43</td>
<td>2.6 × 10⁻⁵</td>
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<tr>
<td>Larvae</td>
<td>ptnp/Fs(3)Apc</td>
<td>600</td>
<td>60</td>
<td>10.0</td>
<td>91</td>
<td>1.9 × 10⁻⁵</td>
</tr>
<tr>
<td>Adults</td>
<td>+/Apc</td>
<td>241</td>
<td>17</td>
<td>5.0</td>
<td>9</td>
<td>0.9 × 10⁻⁵</td>
</tr>
<tr>
<td>Adults</td>
<td>ptnp/Fs(3)Apc</td>
<td>398</td>
<td>20</td>
<td>5.0</td>
<td>40</td>
<td>1.7 × 10⁻⁵</td>
</tr>
</tbody>
</table>

ptnp/Fs(3)Apc and control +/Fs(3)Apc larvae or adults were irradiated with 1500 R of X rays for induction of ptnp/ptnp (+/+ in the control) follicle cell clones through mitotic recombination. Both the Fs(3)Apc mutation and the ptn gene are located on chromosome arm 3L.

* All females were tested for a minimum of 14 days.

* Offspring/female/day.
of $ptn^e$/+ females to $+/+$ males is likely caused by the antimorphic effects of both the maternally provided and the zygotically produced $ptn^e$ mutant product. Viability of $+/ptn^e$ (paternal) embryos produced by $+/+$ females crossed to $ptn^e/+$/males can therefore be explained by the presence of a sufficient amount of maternal $ptn^e$ gene product. These embryos survive but the early effect of $ptn^e$ on chromatin regulation becomes visible by its strong enhancement of PEV and ectopic silencing of mini-white gene inserts as well as induction of ectopic SU(VAR)3-9 binding at >100 sites along all chromosomes. An early zygotic activity of the paternally inherited $ptn^e$ allele is also indicated by lethality of $+/ptn^e$ (paternal) embryos derived from a cross of $ptn^e/+$/females with $ptn^e/+$/males. In all these genotypes, the amount of $ptn$ wild-type product might be reduced far below 50% by the antimorphic nature of the $ptn^e$mutant product. Homozygotes for the recessive $ptn$ mutation produced by a cross of $ptn^e/+$/females with $ptn^e/+$/males or $ptn^e/ptn$ males do not show a dominant enhancer effect on PEV because the maternally provided wild-type gene product is sufficient for normal cleavage and chromatin assembly.

The $ptn^e$ causes ectopic association of heterochromatin protein SU(VAR)3-9 with numerous sites along all euchromatic parts of the Drosophila chromosome complement. Rea et al. (2000) showed a site-specific histone H3 methyltransferase activity of mammalian SU(VAR)3-9 proteins. Overexpression of Su(var)3-9 and SUV39H1 enhances heterochromatin-induced gene silencing in Drosophila and mammalian cell lines, respectively (Tschiersch et al. 1994; Firestein et al. 2000). All these data predict a key role of the SU(VAR)3-9 protein in assembly of higher-order chromatin structure and gene silencing in heterochromatin. The $ptn^e$ mutation might cause nucleation of heterochromatin-like structures at ectopic SU(VAR)3-9 binding sites. Therefore, silencing of mini-white gene inserts observed in $ptn^e/+$/flies might be caused by formation of ectopic heterochromatin. An influence of $ptn^e$ on higher-order chromatin structure in heterochromatic regions is indicated by strong enhancement of PEV. According to this model, the $ptn^e$ mutation might interfere with both chromosomal distribution as well as architecture and function of heterochromatin protein complexes. Embryonic lethality induced by $ptn^e$ might also be caused by its possible regulatory effect on distribution and function of heterochromatin protein complexes. Additive effects of maternally provided and zygotically produced $ptn^e$ mutant product could result in extensive heterochromatization all over the genome. Such a model would also explain the abnormal morphological structure of cleavage nuclei in $ptn^e/+$/embryos and of nurse cell nuclei in egg chambers of mutant heterozygous and homozygous females.

Alternatively, the $ptn$ gene-encoded product might be an abundant chromatin protein associated with eu- and heterochromatic regions and its loss could result in increased accessibility of chromatin for components inducing compaction and gene silencing. As a consequence $ptn^e$ might affect normal transition of cleavage chromatin into chromatin of somatic cells with its subdivision into euchromatic and heterochromatin. If the $ptn$ gene product represents a factor required to maintain an open chromatin conformation, its absence could result in excess chromatin condensation because activities of chromatin condensing factors are no longer balanced. Such an explanation is also consistent with our data showing an early zygotic lethal effect of $ptn^e$. Complete elimination of the $ptn$ gene causes an arrest of development during female germ-line differentiation and its function in early embryonic development can be revealed only by the help of specific types of mutant alleles.

The $ptn^e$-encoded antimorphic mutant gene product might reduce the activity of the $ptn^e$ allele through formation of nonfunctional dimers or by competition for a common interaction partner for heteromer formation. This type of heteromer disruption has been shown in studies of other dominant female-sterile mutations. The mutant tub67C a-tubulin molecules encoded by the Tomaj$^e$ dominant female-sterile alleles disrupt microtubule formation (Mathe et al. 1998) and the importin-β molecules encoded by the Ketel$^e$ dominant female-sterile mutations compete with normal importin-β counterparts for interaction with importin-α (J. Szabad, unpublished data).

Processes of chromatin transition at the end of cleavage, when the cell cycle is prolonged (Edgar and O’Farrell 1989), H1 histones are incorporated into chromatin (Ner and Travers 1994), and heterochromatin is formed (cf. Fox et al. 1993), are only poorly understood.

The $ptn$ gene appears to represent a candidate gene involved in control of euchromatin-heterochromatin balance. The gain-of-function type $ptn^e$ mutation induces strong enhancement of heterochromatin-induced gene silencing in PEV as well as transgene repression within euchromatic regions. It could act in either a structural or regulatory fashion to influence global processes of chromatin regulation during early development. Exceptional gain-of-function alleles such as $ptn^e$ will serve as useful tools for molecular analysis of these processes. This study provides the first step toward the molecular definition of $ptn$ function in the control of chromatin regulation during development of female germ-line cells, the formation of cleavage nuclei chromatin, and its transition into higher-order chromatin structures typical for somatic cells.

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